

Claims

1. A diagnostic method for detecting and identifying bacterial species causing infections from a clinical sample, characterized by
 - a) amplifying DNA isolated from said clinical sample using a mixture of DNA primers that comprises sequences which hybridize with the sequences that originate from conserved regions of genes encoding topoisomerases, especially *gyrB/parE*, of bacterial species causing said infections, said sequences comprising sequences identified with SEQ. ID. NR: 76 and 77 or with complementary sequences thereof or functional fragments thereof,
 - b) contacting the amplified DNA with a desired combination of oligonucleotide probe sequences that hybridize under normal hybridization conditions with hyper-variable regions situated near said conserved regions of genes encoding topoisomerases, especially *gyrB/parE*, of bacterial species causing said infections, said sequences being bacterial species-specific under said hybridization conditions, and
 - c) detecting the formation of a possible hybridization complex.
2. The diagnostic method according to claim 1, characterized in that said infections causing bacterial species are bacterial species that cause respiratory tract infections.
3. The diagnostic method according to claim 1 or 2, characterized in that said hyper-variable region is the hyper-variable region of the gene encoding the *gyrB* and/or *parE* protein of a bacterial species selected from *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Escherichia coli*, *Moraxella catarrhalis*, *Legionella pneumophila*, and *Fusobacterium necrophorum*.
4. The diagnostic method according to any one of claims 1 to 3, characterized in that the length of oligonucleotide probe sequences used in step b) is 15 – 30, more preferably 20 – 30, and most preferably 21 – 25 nucleic acids.
5. The diagnostic method according to any one of claims 1 to 4, characterized in that said combination of oligonucleotide probe sequences comprises all or a portion of the sequences identified with SEQ. ID. NR: 1 to 69, and/or complementary sequences thereof, or functional fragments thereof.

6. The diagnostic method according to claim 5, characterized in that said combination of oligonucleotide probe sequences comprises all the sequences identified with SEQ ID. NR: 1 to 69.

7. The diagnostic method according to any one of claims 1 to 6,
5 characterized in that said combination of oligonucleotide probe sequences is attached onto a solid support.

8. The diagnostic method according to claim 1, characterized in that the DNA isolated from the clinical sample in step a) is amplified using the polymerase chain reaction (PCR) and that the DNA amplified in step b) is con-
10 tacted with bacterial species-specific oligonucleotide probes attached onto a solid support.

9. The diagnostic method according to claim 7 or 8, characterized in that said solid support is treated glass.

10. The diagnostic method according to claim 1, characterized in
15 that suitably labeled nucleotides are used in the amplification of DNA isolated from a clinical sample in step a) to generate a detectable target strand.

11. The diagnostic method according to claim 10, characterized in that the amplified and optionally labeled target DNA in step b) is contacted with a solid support, on which all bacterial species-specific oligonucleotide probes
20 identified with SEQ. ID. NR: 1 to 69 and/or complementary sequences thereof have been attached.

12. The diagnostic method according to claim 10, characterized in that the amplified and optionally labeled target DNA in step b) is contacted with a solid support on which specific oligonucleotide probe sequences detecting
25 one specified bacterial species or a few specified bacterial species causing infections have been attached, said sequences being selected from sequences shown in Tables 4A and 4B and/or complementary sequences thereof.

13. The diagnostic method according to any one of claims 1 – 12, characterized in that the microarray technology is used in step c).

30 14. A DNA primer mixture, characterized by comprising sequences that hybridize with sequences of the conserved regions of genes encoding topoisomerases, especially the *gyrB* and/or *parE* proteins, of bacterial species that cause infections, especially bacterial species that cause respiratory tract infections, said mixture comprising sequences identified with SEQ. ID. NR: 76
35 and 77 and/or reversed or complementary sequences thereof or functional fragments thereof.

15 15. An oligonucleotide sequence useful in the diagnosis of infection causing bacterial species, characterized in that it hybridizes under normal hybridization conditions with a sequence of a hyper-variable region that is bacterial species-specific and is situated near the conserved regions of genes encoding topoisomerases, especially the *gyrB* and/or *parE* proteins, said oligonucleotide sequence being one of the sequences identified with SEQ. ID. NR: 1 to 69 and/or complementary sequences thereof functional fragments thereof.

10 16. The combination of oligonucleotide probe sequences useful in the diagnosis of infection causing bacterial species, characterized by comprising any combination of the sequences identified with SEQ. ID. NR: 1 to 69 and/or complementary sequences thereof or functional fragments thereof.

17. The combination of oligonucleotide probes according to claim 16, characterized by comprising all of the sequences identified with SEQ. ID. NR: 1 to 69.

15 18. The use of the combination of oligonucleotide probes according to claim 16 or 17 for the detection, identification, or classification of infection causing bacterial species.

20 19. A diagnostic kit for use in the diagnosis of infection-causing bacteria, especially those causing respiratory tract infections, characterized by comprising

25 a) a DNA primer mixture comprising sequences that hybridize with sequences of the conserved regions of genes encoding topoisomerases, especially the *gyrB* and/or *parE* proteins, of bacterial species that cause infections, especially bacterial species that cause respiratory tract infections, said mixture comprising sequences identified with SEQ. ID. NR: 76 and 77 and/or complementary sequences thereof or functional fragments thereof of the invention as defined above;

30 b) a combination of bacterial species-specific oligonucleotide probe sequences, optionally attached on a solid support, comprising any combination of the sequences identified with SEQ. ID. NR: 1 to 69 and/or reverse or complementary sequences thereof or functional fragments thereof.

c) positive and optionally negative control probe sequences, and optionally

35 d) reagents required in the amplification, hybridisation, purification washing, and/or detection steps.